Next-Generation TCR Bispecifics (TCER) Targeting Peptide-HLA Antigens for the Treatment of Patients with Solid Tumors

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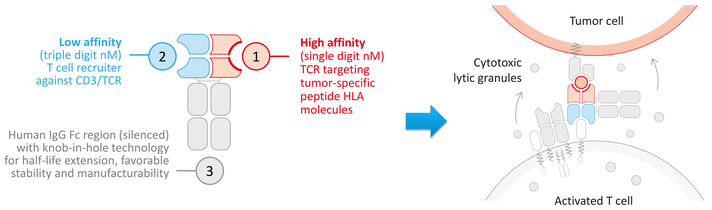
Background – Overcoming Challenges of T cell Engaging Bispecifics

T cell engaging bispecifics have emerged as a promising therapeutic opportunity for patients with solid cancers. However, challenges related to target specificity and drug safety remain and many efforts are being made to generate optimized molecules with improved pharmacodynamics while reducing T cell engager-associated toxicities. We have developed a pipeline of bispecific **T cell engaging receptor (TCER)** molecules comprising a T cell receptor (TCR) for giving access to intracellular tumor antigens presented as peptide-HLA molecules. The next-generation design of TCER is established through a novel, low-affinity T cell recruiting antibody aiming at conferring a favorable drug safety profile while enabling a highly potent anti-tumor response. TCER molecules are further equipped with an effector function-silenced Fc region for prolongation of serum half-life.

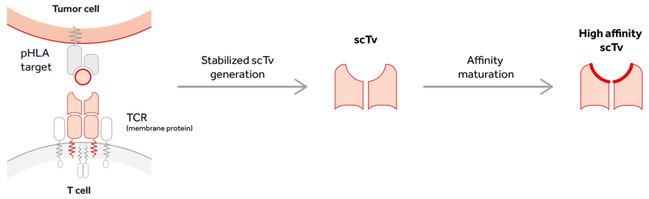
The Next-generation of TCR Bispecifics – TCER



Proprietary TCER format consisting of three distinct elements designed for optimal efficacy and minimal toxicity risk in patients

TCER binds to target, recruits and activates T cells and initiates tumor cell killing

Discovery and Affinity Maturation of TCRs Required for TCER Generation



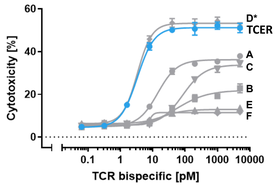
- **XCEPTOR** for TCR Discovery, Engineering & Validation
- Generation of **multiple TCR candidates per target**
- Unique XPRESIDENT-guided **on- and off-target toxicity screening**

- TCR variable domains (scTv format) are **stabilized and optimized** for facilitated production in mammalian cells
- This enables combination with various bispecific platforms

- Low binding affinity of natural TCRs is **increased by at least 1,000-fold** while retaining high specificity
- XPRESIDENT's immunopeptidome database is essential for **specificity-controlled affinity maturation**

TCER Format Exhibits Highest Potency Combined with Specificity in Targeting pHLA Antigens

Killing of target-positive tumor cells by different TCR Bispecifics



*high potency of 2+1 format D was caused by pronounced unspecific reactivity against T2

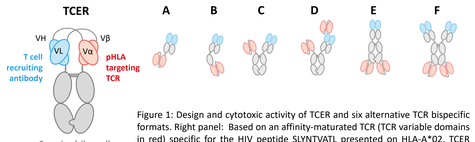


Figure 1: Design and cytotoxic activity of TCER and six alternative TCR bispecific formats. Right panel: Based on an affinity-maturated TCR (TCR variable domains in red) specific for the HIV peptide SLYNTVATL presented on HLA-A*02, TCER and six alternative TCR bispecific formats were generated using the identical Fab region for T cell recruitment (variable Ab domains in blue). Silenced IgG1 Fc domains with knob-into-hole (KiH) were utilized to facilitate heterodimerization. Left panel: PBMC-mediated cytotoxicity of TCR bispecific formats against HLA-A*02–positive T2 cells loaded with HIV peptide. Cytotoxicity was calculated based on LDH release during 24h coculture of PBMC and T2. LDH content of T2 cells alone was used as 100% cytotoxicity reference leading to underestimated cytotoxicity values due to T2 cell proliferation.

Novel, Low-Affinity Recruiter Designed to Improve Efficacy/Toxicity Profile of TCER

TCER molecules are designed with a high affinity TCR and a low affinity T cell recruiting Ab to optimize biodistribution*. The design also intends to selectively induce T cell activation at the tumor site but not in the periphery and thereby reducing immune-related toxicities, like cytokine release syndrome, and to reach sufficiently high drug doses for achieving meaningful clinical responses. * Refer to literature data for other low-affinity recruiters (e.g. Harber *et al.*, 2021, Nature; Trinklein *et al.*, 2019, mAbs)

Superior tumor control using a novel, low-affinity recruiter with high T cell activation capacity

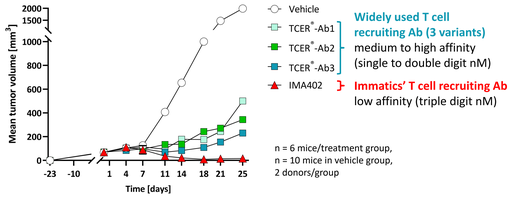


Figure 2. *In vivo* efficacy assessment of TCER molecules incorporating identical tumor-targeting TCR domains, but different T cell recruiting Ab domains in Hs695T (melanoma) cell line xenograft model in NOG mice. Weekly intravenous injections of 0.025 mg/kg body weight of PRAME-specific TCER molecules for three weeks starting at study day 1 after intravenous transfusion of human PBMC. PRAME TCER IMA402 utilizes a novel, low affinity recruiter (triple digit nM affinity) binding both CD3 and TCR. Analogous TCER molecules TCER-Ab1, -Ab2 and -Ab3 utilize medium to high affinity recruiters (TCER-Ab1: 39 nM, TCER-Ab2: 9 nM, TCER-Ab3: 31 nM) binding CD3 only.

Target-unrelated cytokine release in human whole blood is reduced with low-affinity recruiter

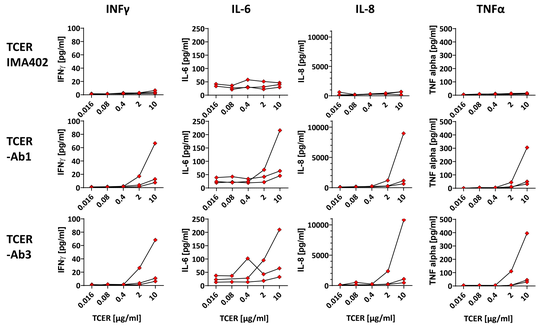


Figure 3. Whole blood cytokine release assay to assess the risk of different recruiters to induce cytokines in absence of target. Recruiter arm-driven non-specific activation of T cells was assessed by measuring TCER-mediated cytokine release in whole blood of 3 HLA-A*02-positive donors and human endothelial cells (HUVEC) after incubation with PRAME TCER IMA402, TCER-Ab1 or TCER-Ab3 for 48h. N = 16 cytokines tested, individual values for 4 exemplary cytokines shown. Higher background of IL-6 is due to the presence of HUVEC. TCER-Ab2 was not tested.

Preclinical Data Package for TCER Programs

- Tumor cell lines presenting target pHLA at endogenous levels
- Tumor cell-mediated cytokine release and proliferation of T cells
- Tumor xenografts in mice
- Pharmacokinetic and -dynamic

- Normal tissue cell types and iPSC-derived normal cells (n≥20)
- Target-negative tumor cell lines
- Alloreactivity screening
- Cytokine release from whole blood
- XPRESIDENT-guided off-target screening based on similarity to target peptide sequence and TCR binding motif

- XPRESIDENT data package
- Absolute quantification of target pHLA copies (AbsQuant)
- Homogeneity of target pHLA presentation within tumors

- Yield and purity of material produced by CHO cells
- Freeze-thaw and storage stress stability
- Sequence liabilities
- N-glycan profiling of TCR domains

Pharmacodynamic and Pharmacokinetic of PRAME TCER (IMA402)

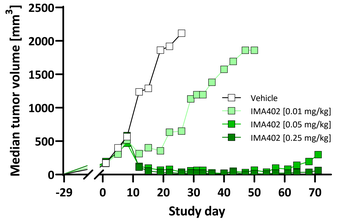


Figure 4. *In vivo* efficacy of IMA402 in large (≈ 195 mm³ average tumor volume) melanoma cell line-derived tumors in MHC I/II knock-out NSG mice over a prolonged observation period of 71 days. Weekly intravenous injections of IMA402 starting at study day 1 after intravenous transfusion of human PBMC. Treatment was discontinued when complete response was noted. Median values for n = 6 mice/group, 2 donors/group.

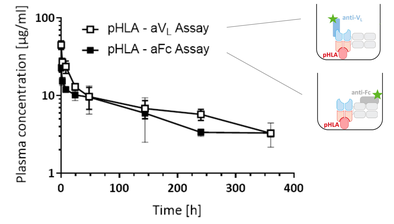


Figure 5. Pharmacokinetic analysis of IMA402 in mice. NOG mice received a single intravenous injection of IMA402 (2 mg/kg). TCER plasma concentrations at different time points were determined by ELISA detecting binding of IMA402 to the PRAME target via pHLA. The integrity of the molecule was confirmed via aVL or aFc detection. Terminal half-life ($t_{1/2}$) was calculated via linear regression of time points between 24 h and 360 h (n=3 per timepoint, mean ± SD).

Manufacturing of PRAME TCER (IMA402)

CMC data support antibody-like manufacturability and developability

- Manufacturing in Chinese Hamster Ovary (CHO) cells able to process natural TCR glycosylation
- High titer (>3.5 g/L) and good stability allowing liquid formulation
- Manufacturing process development completed
- Manufacturing advantages of TCER format: no formation of typical but unwanted Hole-Hole and Knob-Knob side products

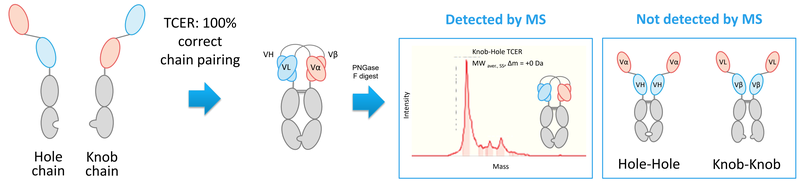


Figure 6. Theoretical product-related impurities such as hole-hole and knob-knob homodimers, are considered inactive due to absence of variable IgG or TCR domains. Total mass analysis by mass spectrometry (MS) of transiently and CHO stably expressed IMA402 has demonstrated lack of hole-hole and knob-knob homodimers in both Protein-L and Protein-A captured TCER fractions. IMA402 was analyzed by MS after complete deglycosylation with PNGase F. With a 100% correct chain pairing of the TCER knob and hole chains, IMA402 can be captured with standard Protein-A chromatography, and no specific downstream processing steps are needed for the removal of these product-related impurities.

Conclusion

T cell engaging receptor (TCER) – a Next-generation TCR Bispecific Format

- Comparison of seven different TCR bispecific formats revealed highest anti-tumor potency for TCER format together with a 2+1 format that failed specificity requirements
- High affinity TCR domains targeting peptide-HLA tumor antigens are generated by specificity-controlled affinity maturation
- Novel, low affinity Ab domains developed for T cell recruitment aim to improve efficacy while minimizing toxicity of TCER molecules as demonstrated in tumor xenograft models in mice and by cytokine release in human whole blood
- IgG Fc region incorporated into TCER format for the extension of serum half-life and improved manufacturability
- Comprehensive preclinical data package including *in vivo* tumor models are generated for each TCER program

PRAME TCER IMA402 with encouraging preclinical and CMC data

- *In vivo* studies in mice demonstrate dose-dependent anti-tumor activity of IMA402 and that sufficiently high drug doses are key to achieving sustained anti-tumor response over prolonged time period
- IMA402 demonstrates a serum half-life of ≈ 8 days in mice suggesting a favorable dosing regimen and prolonged drug exposure at therapeutic levels when compared to TCR bispecifics lacking half-life extension
- TCER IMA402 is manufactured by utilizing standard processes of mAb production resulting in high titer, protein quality and stability while unwanted side products are absent, a unique feature of our TCER format

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